Myeloperoxidase (MPO) Assay Kit

Catalog No.: abx294012

Size: 96 tests

Detection Range: 19.42 U/L – 893.31 U/L

Sensitivity: 19.42 U/L

Storage: Store all components at 4°C in the dark.

Application: For detection and quantification of MPO activity in serum, plasma, milk, cell culture supernatants and tissue samples.

Introduction

Myeloperoxidase (MPO) is a heme-containing cationic glycoprotein that belongs to the heme peroxidase family. MPO is a dimer formed by polymerization of two subunits, with each subunit containing a heavy chain and a light chain. MPO contains anti-microbial properties and plays a role in the generation of oxidants and host defense mechanisms in neutrophilic granulocytes. MPO catalyses the breakdown of Hydrogen Peroxide (H_2O_2) to oxidize chloride and hypochlorous acid, which reacts with o-dianisidine to produce a yellow product.

Abbexa's MPO Assay Kit is a quick, convenient, and sensitive method for measuring and calculating MPO activity. The absorbance should be measured at 460 nm. The intensity of the color is proportional to the activity of MPO which can then be calculated.

Kit components

1. 96-well microplate
2. Assay Buffer: 1 x 20 ml
3. Reagent A: 2 vials
4. Reagent B: 2 vials
5. Saline Solution: 6 ml
6. Clarifying Solution: 2 x 1.2 ml
7. Detection Reagent: 2 vials
8. Substrate: 0.1 ml
9. Acid Reagent: 1 ml
10. Plate sealer: 2

Materials Required But Not Provided

1. Microplate reader (460 nm)
2. Distilled water
3. Pipette and pipette tips
4. Vials/tubes
5. Sonicating water bath
6. Vortex mixer

Protocol

A. Preparation of reagents and samples

1. Reagents

- **Assay Buffer working solution:** Dilute with double distilled water at a ratio of 1:9. Mix thoroughly. Prepare immediately before carrying out the assay. Unused Assay Buffer Working Solution can be stored at 4°C for 2 weeks.
- **Reagent A Working Solution:** Dissolve a vial of Reagent A in 60 ml of pre-prepared Assay Buffer Working Solution to prepare Reagent A Working Solution. Mix thoroughly. Prepare immediately before carrying out the assay. Unused Reagent A Working Solution can be stored at 4°C for 2 weeks.
- **Reagent B Working Solution:** Dissolve a vial of Reagent B in 3 ml of Saline Solution to prepare Reagent B Working Solution. Mix thoroughly. Prepare immediately before carrying out the assay and mix fully. Unused Reagent B Working Solution can be stored at 4°C for 2 weeks.
- **Detection Reagent**: Dissolve a vial of Detection Reagent in 12.5 ml of Assay Buffer Working Solution. Add 12.5 µl of Substrate. Mix thoroughly and prepare immediately before carrying out the assay. Store in the dark at 4°C.

Note: *If the Clarifying Solution is not transparent, incubate at 37°C in a water bath until the solution is clear.*

2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.
The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000 × g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and pretreat immediately, assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using conventional methods. Centrifuge for 10 mins at 1000-2000 × g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and pretreat immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 9 ml homogenization medium. Homogenize by hand on ice, using a mechanical homogenizer, or by ultrasonication. Collect the supernatant, store on ice and pretreat immediately.
- **Milk samples:** Collect fresh milk sample, centrifuge at 4°C for 10 minutes at 10,000 x g. Discard the upper layer and collect the middle layer. Store on ice and pretreat immediately.

Notes:

- Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.
- Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT. Samples should not contain chelating agents such as EDTA
- Human serum, plasma, milk; cell culture supernatant; rat serum, plasma and tissue homogenate samples are recommended to be undiluted.
- Where sample dilutions are required, dilute with Reagent A Working Solution.

3. Sample pretreatment

- **Serum, plasma, cell culture supernatants and milk samples:** Add 45 µl of sample and 45 µl of Reagent A Working Solution to a vial. Mix fully, add 10 µl of Reagent B Working solution, mix and incubate at 37°C for 15 minutes. Take the processed sample for assay immediately.
- **Tissue Homogenates:** Add 90 µl of tissue homogenate and 10 µl of Reagent B Working Solution to a vial. Mix fully, and incubate at 37°C for 15 minutes. Take the processed sample for assay immediately.

B. Assay Procedure

1. Set the Sample and Control tubes. We recommend setting up each sample in duplicate.
2. Add 350 µl of distilled water to the Control tubes.
3. Add 20 µl of Sample to all tubes.
4. Add 20 µl of Clarifying Solution to all tubes.
5. Add 350 µl of pre-prepared Detection Reagent to the Sample tubes.
6. Mix thoroughly and incubate in a water bath at 37°C for 30 minutes.
7. Add 5 µl of Acid Reagent to all tubes.
8. Mix thoroughly and incubate in a water bath at 60°C for 10 minutes. Centrifuge at 3000 × g for 10 minutes and take 300 µl of the supernatant for detection.
9. Measure the OD values at 460 nm with a microplate reader. If precipitation occurs, incubate at 37°C and measure the OD once precipitation dissolves.

C. Calculation of Results

1. Serum, plasma, cell culture supernatants and milk samples:

One unit of MPO activity is defined as the quantity of MPO in 1 L of sample that catalyzes the consumption of 1 µmol/L H_2O_2 at 37°C in 30 minutes.

$$\text{MPO activity(U/L)} = \frac{\Delta A}{11.3 \times b} \times \frac{V_1 \times V_{Total}}{V_{Sample} \times V_2} \times f \times 1000$$

$$= \frac{174.78 \times \Delta A}{V_{Sample}} \times f$$

2. Tissue samples:

One unit of MPO activity is defined as the quantity of MPO in 1 g wet weight of tissue sample that catalyzes the consumption of 1 µmol/L H_2O_2 at 37°C in 30 minutes.

$$\text{MPO activity(U/L)} = \frac{\Delta A}{11.3 \times b} \times \frac{V_3 \times V_{Total}}{V_2 \times W \times 0.9}$$

$$= \frac{1.942 \times V_3 \times \Delta A}{W}$$

where:

ΔA	OD value of the sample ($OD_{Sample} - OD_{Control}$)
b	optical path of cuvette, 1 cm
V_{Total}	Total volume of the reaction system, 0.395 ml
V_{Sample}	Volume of sample added in sample pretreatment for serum, plasma, and milk, 0.045 ml
V_1	Total volume in sample pretreatment step, 0.1 ml
V_2	Volume of sample added to the reaction system, 0.02 ml
V_3	Volume of Reagent A working solution added to tissue sample (e.g. 10 µl)
f	dilution factor of the sample prior to assay
W	wet weight of sample (g)
11.3	Constant
1000	Conversion factor (1 L = 1000 ml)
0.9	Ratio of sample volume to total volume in sample pretreatment (0.09 ml / 0.1 ml = 0.9)